

Evaluation of Diuretic Activity of Ethanolic Extract of *Habb-e-Kaknaj* (*Physalis alkekengi* Linn. Fruit) in Rat

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Abstract

The present study was carried out to evaluate the diuretic activity of ethanolic extract of *Habb-e-Kaknaj* on healthy Wistar rats of either sex. The animals were kept on fasting for 8 hrs; thereafter they were administered normal saline (30 ml/kg) and divided into three groups of six animals each. Animals in group I-III were treated with distilled water, furosemide (4 mg/kg) and ethanolic extract of *Habb-e-Kaknaj* (450 mg/kg), respectively. After respective treatment all the animals were kept separately in metabolic cages and the urine passed by them during a period of 6 hours was collected. In standard control and the test group the urine volume and urinary sodium and potassium level increased significantly when compared with plain control. The study demonstrated that ethanolic extracts fruits of *Physalis alkekengi* Linn. possesses significant diuretic effect.

Keywords: *Mudirre bol*, *Kaknaj*, Furosemide, Diuretic, *Physalis alkekengi*, Unani medicine

Introduction

Kidney is designed to filter a large quantity of plasma. It reabsorbs those substances that the body must conserve, and leaves behind and/or secretes substances that must be eliminated. Diuretics increase the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis etc (Gilman et al., 2001). A number of diuretics such as mannitol, thiazides, furosemide, and ethacrinic acid are widely used clinically (Singh et al., 1991). Diuretics specially, thiazides and furosemide have been associated with numerous adverse effects, such as electrolyte imbalance, metabolic alterations, development of new onset diabetes, activation of the renin-angiotensin and neuroendocrine systems, and impairment of sexual function (Gupta and Neyses, 2005). A number of plant drugs on the other hand are used in traditional medicines in the management of renal diseases and other associated disorders without any major side effect. Most of such drugs are considered therapeutically important because of their attributed diuretic effect, and few of them have been reported in certain scientific studies to possess significant diuretic activity (Caceres et al., 1987; Lu Wei et al., 1994; Hemamalini and Varma, 2007).

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An important plant drug of Unani Medicine which is frequently used in different renal diseases and oedematous conditions is *Kaknaji* (*Physalis alkekengi* Linn. Family-Solanaceae). It is a diffuse perennial herb about 80 cm (32 inches) in height (Anonymous, 1996). It is considered to be originally native of the region extending from Japan to South East Europe and is naturalized in many countries (Anonymous, 1992; Nadkarni, 1954). It comprises 100 species of which only three species are native to India (Bhattacharji, 2004). The fruits of the taxon are reddish or orange red in colour, fully covered in a thin sheath of membrane giving it a shape of urinary bladder and contain large number of flattened reniform seeds of light brown colour (Singh, 1974). They are very juicy and have an acidulous bitter test. The fruits of the plant are commonly used from the ancient time in Unani system of medicine, as diuretic (*mudirre bol*) (Aawan, 1993; Aziz, 1948; Ghani 1920; Husain, 1872; Karim, 1888; Khan, 1313H; Nabi, 1901) in different pathological conditions such as in urinary tract infection (Aziz, 1948; Ibn Sina 1906; Karim, 1888; Khan, 1313H), wound of urinary tract (Ibn Sina 1906; Ghani 1920; Husain, 1872), wound of kidney and urinary bladder (Aawan, 1993; Aziz, 1948; Ghani 1920; Karim, 1888; Nabi, 1901), cystitis (Khan, 1313H; Karim, 1888; Aziz, 1948), kidney stone (Aawan, 1993) and other diseases of kidney and urinary bladder (Ghani 1920; Karim, 1888; Nabi, 1901). It has also been described to be anti-inflammatory (Aawan, 1993; Ghani 1920; Khan, 1313H), lithotryptic (Kabiruddin, ynm), nephroprotective (Kabiruddin, ynm; Karim, 1888; Khan, 1313H), anthelmintic (Karim, 1888; Khan, 1313H; Ghani, 1920; Nabi, 1901), contraceptive (Karim, 1888; Khan, 1313H), and hepatoprotective (Khan, 1313H; Karim, 1888; Kabiruddin, ynm). Its use as a diuretic agent has also been described in ethnobotanical literature (Anonymous 1992; Anonymous, 1996; Chopra, 1956; Dymock, 1891; Khory & Katrak, 1985; Lindley, 1981; Nadkarni, 1954; Singh, 1974; Evans, 2002). In certain recent studies its nephroprotective (Wasim et al., 2010), steroidal & metabolic activity (Ghufran et al., 2012) and antineoplastic activities (Dornberger, 1986) have been reported. But probably no pharmacological study has been carried out so far to confirm its diuretic effect. In present study therefore an attempt has been made to evaluate the diuretic activity of ethanolic extract of *Habb-e-Kaknaji* in albino rats.

Materials and Methods

Plant material

The fruits of *Physalis alkekengi* Linn. (*Habb-e-Kaknaji*) were procured from Dawakhana Tibbiya College, Aligarh Muslim University (AMU), Aligarh, India. Professor S. H. Afaq and Dr. M. Inamuddin, pharmacognocists at Department

of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh authenticated the drug sample. The voucher specimen No. WA/2005/3 has been deposited in museum of the Dept. of Ilmul Advia, AMU, Aligarh for future reference.

Preparation of extract

The fruits of *Kaknaj* were dried at room temperature and powdered in an electrical grinder. The powdered drug in a known quantity was immersed in 70% ethanol (ethanol and distilled water 7:3) in the ratio of 1:5 (drug + solvent) and left for 12 hours at room temperature and then extracted for 6 hours in Soxhlet's apparatus at a fixed temperature of 80 °C. The filtrate, after filtration through filter paper was dried over water bath. The yield percentage was calculated with reference to the weight of crude drug and was found to be 30% w/w.

Dosage of the drug

Dose of albino rats was calculated by multiplying the human therapeutic dose, described and practiced in Unani Medicine (Aawan, 1993; Ghani, 1920; Husain, 1872; Khan, 1313H; Nabi, 1901; Singh, 1974) by conversion factor of 7 (Freidrich, 1968). The dose of the extract was determined with reference to the dose of crude drug and was found to be 450 mg/kg.

Animals

The study was carried out on healthy Wistar albino rats of either sex weighing 100-150 gm, divided into three groups of six animals each. The animals were acclimatized to the laboratory condition for 5 days before the experiment. They were housed in clean polypropylene cages and kept under standard environmental condition; at a temperature of 23^o± 2 °C with 12 hrs light and dark cycle. They were fed standard food pellets and water *ad libitum* unless stated otherwise.

Diuretic activity

The diuretic activity of *Habb-e-Kaknaj* (*Physalis alkekengi* Linn. fruits) was assessed by the method of Lipschitz et al. (1943), Taylor and Toplis (1962), Amin et al., (1994) and Afzal et al., (2004). Wistar rats of either sex were divided into three groups of six animals each. The animals were deprived of food and water for 8 hours prior to the experimentation. Thereafter, all the animals were administered 30 ml/kg of (0.9% NaCl) normal saline orally. After administration of the normal saline, the animals of group I were given single

dose of 1 ml of distilled water orally and served as plain control. The animals of group II were treated with single dose of furosemide 4 mg/kg, orally, (dissolved in 1 ml distilled water) and served as standard control, while the animals of group III were treated with ethanolic extract of *Habb-e-Kaknaj* in the dose of 450 mg/kg, (dissolved in 1 ml of distilled water) orally and served as test group. Immediately after the treatment of test and standard drug all the animals were kept separately in metabolic cages. The mineral oil (Paraffin oil) was applied on the upper surface of the bottom of metabolic cages. A glass funnel kept under the metabolic cage, was also lubricated with the mineral oil. The purpose of application of mineral oil was the prevention of urine loss through evaporation. Urine was collected after 6 hours of the treatment. Further, the bladder was emptied by pulling the base of tail of each rat (Radhika et al., 2010).

Urine Analysis

The parameters observed were total urine volume and the concentration of sodium and potassium levels excreted in it. The concentration of sodium, and potassium chloride were determined by using 'flame photometer'.

Statistical Analysis

The results were given as mean \pm S.E.M. Significance was determined by using the Student's 't' test. *P*-value equal to or less than 0.05 showed significance.

Results

Ethanol extract of fruit of *Physalis alkekengi* at a dose of 450 mg/kg induced significant ($P<0.001$) increase in urine volume, as compared to plain control group. Ethanol extract of test drug was found to produce significantly ($P<0.001$) increased natriuresis and kaliuresis as compared to plain control group. In plain control group, the mean volume of urine was found to be 0.76 ± 0.033 ml, while in standard control (treated with furosemide) it increased significantly to 3.43 ± 0.102 ml ($P<0.001$). In test group, the volume of urine collected during 6 hrs increased significantly to 2.55 ± 0.042 ml ($P<0.001$), when compared with plain control. The mean sodium level in plain control was found to be 1887.33 ± 1.764 ppm. In case of standard control and test group, sodium levels were found to be 2227.50 ± 0.885 ppm ($P<0.001$) and 2166.16 ± 0.477 ppm ($P<0.001$), respectively. The concentration of potassium was calculated to be 462.50 ± 0.619 ppm in plain control group and 670.33 ± 0.557 ppm ($P<0.001$) and 650.33 ± 0.494 ppm ($P<0.001$) in standard and test group, respectively. (Table 1)

Table 1: Diuretic activity of ethanolic extract of *Habb-e-Kaknaj* (*Physalis alkekengi* Linn.)

Groups	Urine volume (ml) (Mean±S.E.M.)	Sodium excretion (ppm) (Mean±S.E.M.)	Potassium excretion (ppm) (Mean±S.E.M.)
Group I (control)	0.76±0.033 y ³ z ³	1887.33±1.764 y ³ z ³	462.50±0.619 y ³ z ³
Group II (standard)	3.43±0.102 z ³	2227.50±0.885 z ³	670.33±0.557 z ³
Group III (test)	2.55±0.042	2166.16±0.477	650.33±0.494

n = 6

1 = P < 0.05 2 = P < 0.01 P < 0.001 3 = P < 0.001

x = against control group

y = against standard group

z = against test group

Discussion

The study showed that ethanolic extract of *Habb-e-Kaknaj* induced an increase in total urine output. It also increases the excretion of sodium and potassium significantly demonstrating that the test drug possesses significant diuretic, natriuretic and kaliuretic effect. Since diuretics increase the rate of urine flow and sodium excretion, and are used to adjust the volume and composition of body fluid in a variety of clinical situations therefore the significant diuretic activity as demonstrated in the present study will be useful in a number of pathological conditions. The therapeutic application of this drug by physicians of Unani medicine in inflammatory and volume overload conditions appears to be justified. *Habb-e-Kaknaj* (*Physalis alkekengi* Linn. fruit) is a well-known diuretic drug (Aawan, 1993; Aziz, 1948; Ghani 1920; Husain, 1872; Karim, 1888; Khan, 1313H; Nabi, 1901), used in Unani Medicine from ancient times. Unani literature describes its use in urinary tract infection (Aziz, 1948; Ibn Sina 1906; Karim, 1888; Khan, 1313H), wound of urinary tract (Ibn Sina 1906; Ghani 1920; Husain, 1872), wound of kidney and urinary bladder (Aawan, 1993; Aziz, 1948; Ghani 1920; Karim, 1888; Nabi, 1901), cystitis (Khan, 1313H; Karim, 1888; Aziz, 1948), kidney stone (Aawan, 1993) and diseases of kidney and urinary bladder (Ghani 1920; Karim, 1888; Nabi, 1901) etc. Present study validated the diuretic effect of kaknaj and its therapeutic use in different diseases as practiced by Unani physicians.

Diuretics are frequently used to relieve pulmonary congestion and peripheral edema. They are also useful in reducing the syndrome of volume overload, decreasing cardiac workload, oxygen demand and plasma volume and thus help decrease the blood pressure (Hoeland et al., 2000). The control of plasma sodium is important in the regulation of blood volume and its pressure, and the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscle (Guyton et al., 1998). The loss of potassium increases when sodium reaches the collecting duct indicating decreased absorption of sodium in earlier part of nephron as occurs with thiazide and loop diuretics. A significant increase in the concentration of Na^+ and K^+ , apart from indicating the relationship between the two also points towards the likely mechanism of action of the test drug (Harvey, et al., 2006). The study indicates therefore that the diuretic effect of the test drug may be caused by the action of the test drug at the thick Ascending Limb like furosemide and/or at Distal Convoluted Tubule like the thiazides. The regulation of sodium potassium balance is also intimately related to renal control of acid base balance. The test drug by increasing the urine volume and inducing natriuretic and kaliuretic effect clearly indicates that it has wide therapeutic potential and can be used in a number of acute and chronic diseases such as pulmonary oedema, general oedema, ascites, portal hypertension, splenohepatomegaly, congestive heart failure, nephrosis, nephritic syndrome and many other diseases etc. Its diuretic effect which was almost equal to that of furosemide warrants that it should be studied further at different dose levels and in diseases of diverse aetiology where diuretics play a role. Some of the important constituents such as physalin, histonin, quercitin (Rastogi & Mahotra, 1998) mineral salts, vit C, citrone and glycol alkaloids etc (Anonymous, 1992, 1996) which have been investigated for some other activities but may also have role in inducing diuresis, as many constituents of plant drugs such Flavonoids, saponins, sterols and triterpenes are known to possess diuretic activity (Chandra et al., 2008).

Conclusion

On the basis of the above findings, it can be concluded that ethanolic extract of *Habb-e-Kaknaj* (*Physalis alkekengi* Linn. fruit) has significant diuretic activity thus supporting its use as a diuretic in a number of renal and other volume overload related disorders by Unani physicians.

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